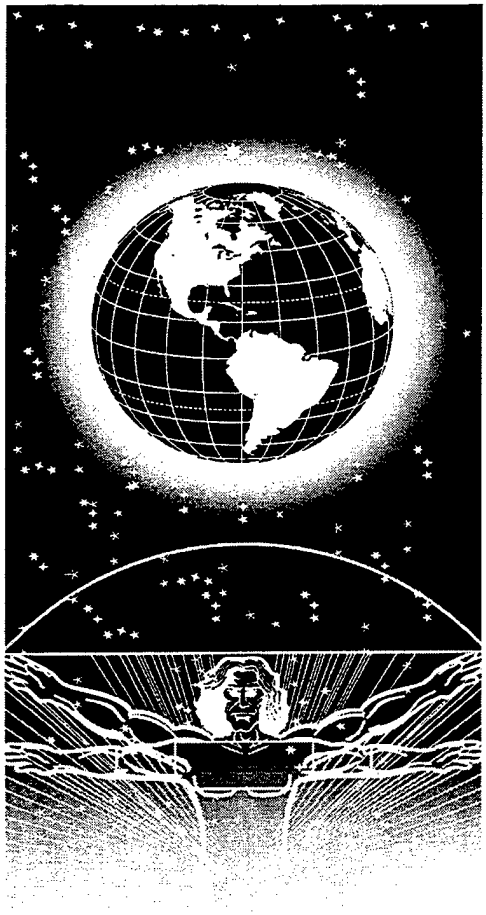
AFRL-HE-WP-TR-1999-0022



UNITED STATES AIR FORCE
RESEARCH LABORATORY

REPEATED DOSE SKIN IRRITATION STUDY ON JET FUELS - A HISTOPATHOLOGY STUDY

William Baker
Thomas Miller

Darol Dodd
James McDougal

MANTECH GEO-CENTERS JOINT VENTURE
P O BOX 31009
DAYTON, OH 45437-0009

March 1999

Final Report for the Period 1 June 1998 to 1 June 1999

Human Effectiveness Directorate
Deployment and Sustainment Division
Operational Toxicology Branch
2856 G Street
Wright-Patterson AFB OH 45433-7400

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Air Force Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Service
8725 John J. Kingman Rd., Ste 0944
Ft. Belvoir, Virginia 22060-6218

DISCLAIMER

This Technical Report is published as received and has not
been edited by the Technical Editing Staff of the Air Force Research Laboratory.

TECHNICAL REVIEW AND APPROVAL

AFRL-HE-WP-TR-1999-0022

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

STEPHEN R. CHANNEL, Maj, USAF, BSC
Branch Chief, Operational Toxicology Branch
Air Force Research Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 1999	Final Report - June 1998-June 1999

4. TITLE AND SUBTITLE
Repeated Dose Skin Irritation Study on Jet Fuels - A Histopathology Study

5. FUNDING NUMBERS
Contract F41624-96-C-9010
PE 62202F
PR 7757
TA 7757A1
WU 7757A114

6. AUTHOR(S)
W. Baker, J. McDougal and T. Miller

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ManTech Geo-Centers Joint Venture
PO Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Human Effectiveness Directorate
Air Force Research Laboratory
Wright-Patterson AFB, OH 45433-7400

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
AFRL-HE-WP-TR-1999-0022

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

JP-8 is the battlefield fuel for DoD and NATO countries. It's use is projected beyond 2025, with employment of additive packages to meet new weapon systems' requirements. One additive package (JP-8+100) currently in use increases the thermal stability of the fuel by 100 F. Questions have been raised about human helath implications of occupational exposures to JP-8, as compared to the phased out JP-4, and to possible differences between JP-8 and JP-8+100. This study investigated the histopathologic effects of daily, topical, dermal exposure to JP-8+100, JP-4 and JP-8 in rats. Full thickness samples of control and treated skin were taken at weekly intervals during the 4-week exposure phase and the 3-week recovery phase. Proliferative, degenerative, and inflammatory changes within the epidermis and dermis were assessed and graded. Mean scores during the exposure phase (69.3 $\pm$ 24.4, 58.9 $\pm$ 7.8, and 69.5 $\pm$ 20.0 for JP-8+100, JP-4, and JP-8, respectively) were significantly different from mean control scores of 16.8 $\pm$ 3.6 ($P<0.0001$). Treatment group scorces did not differ significantly from one another. Characteristic of all treatment groups was the rapid reversibility of the lesions following withdrawal from exposure. Mean scores during the recovery phase (20.1 $\pm$ 4.2, 19.7 $\pm$2.7, and 21.6 $\pm$7.0 for JP-8+100, JP-4, and JP-8, respectively) did not differ from control scores.

14. SUBJECT TERMS				15. NUMBER OF PAGES
JP-8	JP-8+100	Skin	Irritation	29
Pathology	Dermal Exposure			16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

THIS PAGE INTENTIONALLY LEFT BLANK.

TABLE OF CONTENTS

SECTION **PAGE**

LIST OF TABLES

LIST OF FIGURES

REPEATED DOSE SKIN IRRITATION STUDY ON JET FUELS – A HISTOPATHOLGY STUDY

INTRODUCTION

JP-8 is the battlefield fuel for DoD and NATO countries. Its use is projected beyond 2025, with the use of additive packages to the parent JP-8 fuel to meet new weapon systems' requirements. One of these changes already in use operationally is an additive package that increases the thermal stability of the fuel by 100°F, referred to as JP-8+100. Questions have been raised about the human health implications of occupational exposures to JP-8, as compared to the phased out JP-4 and to possible differences between JP-8 and JP-8+100. Efforts are underway within the Air Force to establish a unified research approach to evaluate the environmental, safety, and occupational health (ESOH) issues of JP-8 fuel and proposed JP-8 additive packages. The Operational Toxicology Branch of the Human Effectiveness Directorate (HEST) is tasked with addressing toxicity issues. Populations most likely to have repeated skin exposure to JP-8 and JP-8+100 are those in refueling operations, bulk storage and distribution, jet engine repair, ground fuel systems maintenance and aircraft fuel cell maintenance and repair. Clinical observations indicate that repeated skin contact results in acute redness and itching or dermatitis. No detailed histopathologic information is available on the effect of repeated skin contact with JP-4, JP-8, or JP-8+100.

Study Objective

The purpose of this investigation is to determine and evaluate the potential of JP-8 and JP-8+100 to produce skin irritation (and toxicity) in rats following repeated short-term skin applications. For comparison of potency, this study will also determine and evaluate the skin irritancy potential of JP-4 following repeated short-term skin applications. As determined in a preliminary dose range finding study (Baker *et al.*, 1999), the most critical endpoint in the study will be histopathologic evaluation of the treated skin. Additionally, post-treatment recovery of skin irritant effects produced by these jet fuels will be determined.

Background

Mammalian toxicity studies on fuels performed by the Toxic Hazards Research Unit at Wright Patterson AFB include acute, chronic toxicity and oncogenic inhalation studies with hydrazine, JP-4, JP-8, JP-8+100, quadricyclane, RP-1 and JP-10. Previous dermal toxicity evaluation studies using JP-8 or JP-8+100 were performed in the rabbit or guinea pig to determine acute dose lethality (LD_{50}), skin irritation or dermal sensitization. Wolfe et al., 1996, carried out acute oral (gavage), dermal, and inhalation (vapor and aerosol) tests with JP-8 and two JP-8+100 jet fuels (Betz and Mobil). No signs of toxic stress were observed in the rat oral tests, and single treatments of 0.5 mL neat jet fuel to rabbit skin produced negative results for skin irritation. Jet fuel failed to elicit a sensitization response following repeated applications on guinea pigs. Acute vapor inhalation studies in rats did not indicate differences in toxic potency between JP-8 and the two JP-8+100 jet fuel additives.

None of these studies were designed to determine and evaluate the irritant effects of JP-8 (or JP-8+100) following repeated skin application. The LD_{50} and skin irritancy tests involved single application of each test substance. The guinea pig sensitization test involved a series of four (0.1 mL) jet fuel applications, including injection of an adjuvant during the third application, over a 10-day period to assess allergenic potential following a challenge exposure (two weeks later) with the test substance. The purpose of the skin sensitization test is to determine if there is an immunologically mediated cutaneous reaction to a substance. In a preliminary dose range finding study (Baker *et al.*, 1999), JP-4 and JP-8 were applied either once or twice daily to the skin of experimental animals for a period of 7 days. A range of skin response scores was determined, primarily on the basis of histopathological evaluation. In general, JP-4 was less irritating than JP-8 and JP-8+100.

The Air Force supports or has supported studies that are evaluating the immunotoxicological potential of aerosolized JP-8 jet fuel (Harris, 1995), the effects of chronic aerosolized JP-8 jet fuel exposure on the lungs and secondary organs (Witten, 1994), and the effects of hydrocarbons on the histologic structure of the male rat kidneys (Eurell, 1994).

MATERIALS AND METHODS

Test Materials

The test substances, JP-4, JP-8, and JP-8+100 were supplied by the Air Force Research Laboratory (AFRL), Propulsion Directorate, Fuels Branch. Pertinent military specifications (MIL-SPEC) are MIL-PRF-5624S, 22 Nov 96 (JP-4); MIL-T-83133D, 29 Jan 92 (JP-8); and MIL-T-83133D, Amendment 1, 19 Sep 95 (JP-8+100). The starting fuel for this experiment was Jet A-1 (POSF3404). JP-8 was produced by addition of an additive to the POSF3404 according to specification POSF3509. JP-8+100 was produced by addition of an additive to the POSF3509 according to specification POSF3509+100. See Table 1a for a description of additives and their quantities. The test substances were analyzed for certain specification tests prior to the initiation of the study (Table 1b) [U.S. Air Force, 1992]. Test substances were stored in a flammable liquid storage cabinet under ambient conditions. The letters A, B, and C were assigned to test substances, JP-8+100, JP-4, and JP-8, respectively, for the purpose of maintaining a nonbiased study design during the antemortem phase of the investigation.

Table 1a. Aviation Turbine Fuels Additives

	Additive	Use	Quantity	Jet A-1	JP-8	JP-8 + 100
1	DIEGME	Ice inhibitor	0.1 vol/vol %	Optional	Required	Required
2	Stadis 450	Static inhibitor	2 mg/L	Optional	Required	Required
3	DCI-4A	Corrosion inhibitor	15 mg/L	None	Required	Required
4	Antioxidant	Inhibits gum formation	25 ppm	Optional	Optional	Required
5	Metal Deactivator	Controls metal catalyzed fuel deterioration	3 ppm	Optional	Optional	Required
6	Detergent/ Dispersant	Cleans engine/ Minimizes particle size	70 ppm	None	None	Required

1. Diethylene glycol monomethyl ether.
2. Proprietary composition
3. Octel (proprietary) product, other manufacturers available
4. N,N-diisopropylparaphenylene diamine or various blends of hindered phenols (e.g.. 2,6-ditertiarybutyl phenol).
5. N,N-disalicylidene-1,2-propanediamine or N,N-disalicylidene-1,2-cyclohexanediamine.
6. Polybutenyl succinimide or other proprietary compositions.

Note: 4,5 and 6 are currently produced by Betz as 8Q462 (proprietary) as an additive package

Table 1b. Fuel Specification Results.

Fuel	Method	Test	MIL-T-83133D 29 Jan 92	Test Result
POSF3404 (JP-8)	D1319	Aromatics, % vol	25 max	18
	D86	Distillation		
		IBP	Report	159
		10 % recovered, °C	205 max	183
		20 % recovered, °C	Report	190
		50 % recovered, °C	Report	208
		90 % recovered, °C	Report	246
		End Point, °C	300 max	266
		Residue, % vol	1.5 max	1.3
		Loss, % vol	1.5 max	1.1
	D56	Flash Point, °C	38 min	52
	D445	Viscosity @ -20°C, cSt	8.0 max	5.2
	D3343	Hydrogen Content, % wt		13.8

Table 1b. Fuel Specification Results (continued)

POSF3121	D3242	Total Acid Number, mg KOH/g	0.015	0.008

(JP-4)	D1319	Aromatics, % vol	25.0	9.7
	D1319	Olefins, % vol	Report	0.5
	D3227	Mercaptan Sulfur, % wt	0.002	0.000
		Total Sulfur, % wt	0.40	0.0187
	D2887	Distillation		
		IBP	Report	73
		10 % recovered, °C	Report	103
		20 % recovered, °C	100 min	111
		50 % recovered, °C	125 min	143
		90 % recovered, °C	Report	221
		End Point, °C	270 max	247

Laboratory Animals and Animal Husbandry

Rats were selected for this investigation because of the experience and historical database of this species in dermal absorption studies (both *in vitro* and *in vivo*) in this laboratory (AFRL/HEST). Forty-two male Fischer rats [CDF®(F-344)/CrlBR], Charles River Laboratories, Raleigh, NC, weighing between 219 and 251 grams, were used. Serology and pathology evaluations indicated the animals were healthy and free of disease. Routine animal husbandry procedures were performed by AFRL/HEST personnel using Standard Operating Procedures for rodents. The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (NRC, National Academy Press, 1996). The rats were housed in plastic shoe-box cages with bedding (one/cage). A 12-hr light/dark cycle was provided. Temperatures were maintained between 72 and 82°C, and relative humidity was maintained between 30 and 84%. Food (Formulab Rodent Diet, PMI Feeds, Inc., St. Louis, MO) and water were available *ad libitum*.

Experimental Design

Group assignment, jet fuel treatment, and schedule of animal termination are given in Table 2. There were no control animals, but untreated dermal sites on experimental animals served as control areas (Table 3).

Table 2. Group Assignment, Treatment, and Schedule of Animal Termination

Group*	Total Number of Animals	Number of Animals Terminated after 7, 14, 21, and 28 Days of Dosing	Number of Animals Terminated after 7, 14, and 21 Days of Recovery (following 28 days of dosing)
A	14	2	2
B	14	2	2
C	14	2	2

*A = JP-8+100
B = JP-4
C = JP-8

Table 3. Number of Control and Treated Dermal Samples

Collection Time	Controls*	JP-8+100	JP-4	JP-8
Study Day 7 (Week1)	6	2	2	2
Study Day 14 (Week 2)	6	2	2	2
Study Day 21 (Week 3)	6	2	2	2
Study Day 28 (Week 4)**	6	2	2	2
Study Day 35 (Week 5)	6	2	2	2
Study Day 42 (Week 6)	6	2	2	2
Study Day 49 (Week 7)	6	2	2	2

*Control samples came from untreated side of each treated animal
**Last day of dosing was Study Day 27

General Procedures and Experimental Evaluations

A volume of 0.156 ml of each test substance was applied (neat) once daily (a.m.) to the skin of experimental animals for a period of 7 to 28 days. The site of application was not wrapped, but animals wore neck collars to avoid grooming of the dosing area. A description of the neck collars has been reported (Baker *et al.*, 1999). An area of 2.5 cm x 5 cm (12.5 cm^2) was chosen as an appropriate surface area for dosing laboratory rats (Baker *et al.*, 1999). This area was measured on the dorsal side of the rat, using the midline as one of the 5 cm sides, and outlined using a felt tip marker. A hand-held pipetter was used to draw the test substance from its container. During each application, the technician carefully dispensed the test substance as evenly as possible over the entire surface area. The skin at the site of test substance application was not abraded. Once-a-week, careful hair clipping procedures were carried out to avoid skin irritation. One person did hair clipping to standardize the process.

On each animal, an anatomically comparable, non-dosed area of skin was used for control. This untreated area was on the dorsal side of the rat, immediately opposite of the midline that served as a border for the treated area.

Clinical Observations and Body Weights

The degree of skin irritation was observed each morning prior to application. Animals were also observed for signs of stress (due to the wearing of neck collars) and health status. Animals were weighed daily prior to dosing without removing neck collars. [Note: Collar weights were not subtracted from the body weight data, because the contribution of a collar weight was considered insignificant to the animal's body weight.]

Scoring the skin sites (treated vs. untreated) for signs of irritation followed the "standard procedure for evaluation of skin reactions" described by Draize and coworkers (1944) and recommended by most regulatory authorities (e.g., EPA) in their guidelines on the conduct of animal tests for skin irritation. Scoring for flaking and lichenification fissure formation was also performed.

The scoring and evaluation of gross skin lesions were based on the follow criteria.

Erythema (Redness)	
No Erythema	0
Very Slight Erythema (barely preceptible –light pink)	1
Well-defined Erythema (dark pink)	2
Moderate Erythema (light red)	3
Severe Erythema (extreme redness)	4
Edema (Swelling)	
No Edema	0
Very Slight Edema (barely preceptible)	1
Slight Edema (edges of area well-defined by definite elevation)	2
Moderate Edema (raised approximately 1mm)	3
Severe Edema (raised >1mm and extends beyond area of exposure)	4
Flaking/Lichenification (thickening of skin) Fissure Formation	
No Changes	0
Drying of Skin with Flaking (present or absent)	A
Lichenification (present or absent)	*

Fissure Formation (vertical splits in epidermis
w/ multifocal pinpoint, black foci [hemorrhage])

Minimal	5
Mild	10
Moderate	15

Termination and Gross Necropsy

At termination, animals were subjected to gross necropsy following CO_2 inhalation overdose. For each animal, skin from the entire treated and untreated area was collected and fixed in 10% buffered formalin for at least 24 hours. The liver, kidneys, and lungs were trimmed, weighed wet, and preserved in buffered formalin.

Histopathology and Methods of Assessment

Following fixation, skin samples were processed using standard protocols for paraffin embedding, sectioning, mounting, and hematoxylin and eosin staining. Microscopic slides were assessed in random order and scored according to the following system.

Erosion: 0=normal, 1=focus limited to region above a dermal papilla, 2=region over several hair follicles or foci above multiple papillae <25% of surface area, 3=extending over 25-50% of section, 4=>51% of section affected

Ulceration: 0=normal, 1=focus limited to region above a dermal papilla, 2=region over several hair follicles or foci above multiple papillae <25% of surface area, 3=extending over 25-50% of section, 4=>51% of section affected

Crust formation:
0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of surface

Epidermitis: 0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of surface

Necrosis: 0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of surface

Acantholysis: 0=normal, 1=1-4, 2=5-8, 3=9-11, 4=>12 foci

Spongiosis: 0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of epidermis

Hydropic Degeneration:
0=absent, 1=present in scattered single cells, 2= present in scattered single cells and in clusters

Orthokeratotic hyperkeratosis:
0=normal, 1= 2x normal, 2=3x normal, 3=4x normal, 4=>4x normal

Parakeratosis: 0=normal, 1=present (minimum/several layers w/nuclei), 2=present & >minimum

Hyperplasia: 0=normal, 1=2-3x normal, 2=4-5x normal, 3=6-7x normal, 4=>7x normal

Hypergranulosis:
0=normal, 1=present

Dyskeratosis: 0=normal, 1=present

Inflammatory infiltrates:
0=normal, 1=scattered single cells, 2=clusters of cells, 3=coalescing clusters, 4=regionally diffuse infiltrates

Edema: 0=normal, 1=single focus within a dermal papilla, 2=several papillae, adjacent or separate w/ foci, 3=focus/foci filling papillae, 4=coalescing foci of edema

Vasodilation: 0=normal, 1=single, visible vessels, 2=several barely dilated vessels single or in cluster, 3=clusters of moderately dilated vessels , 4=numerous widely dilated vessels

Direct quantitative observations for epidermal thickness are expressed in micrometers. Epidermal epithelial cell counts and counts of mitotic figures were made within the limitations stated on the assessment sheet.

Statistics (Skin Histopathology)

For statistical comparisons, the 42 control observations were combined for increased statistical power. The thrust of this study is to assess and compare gross and microscopic dermal changes during the exposure and the recovery phases of the experiment. For these statistical comparisons, the 2 observations per week per exposure phase group (n=8), and as well the recovery phase observations (n=6) were separately pooled. These data were statistically analyzed using a t-test or the Mann-Whitney Rank Sum Test, test capable of adjusting for the unequal numbers of observations.

RESULTS AND DISCUSSION

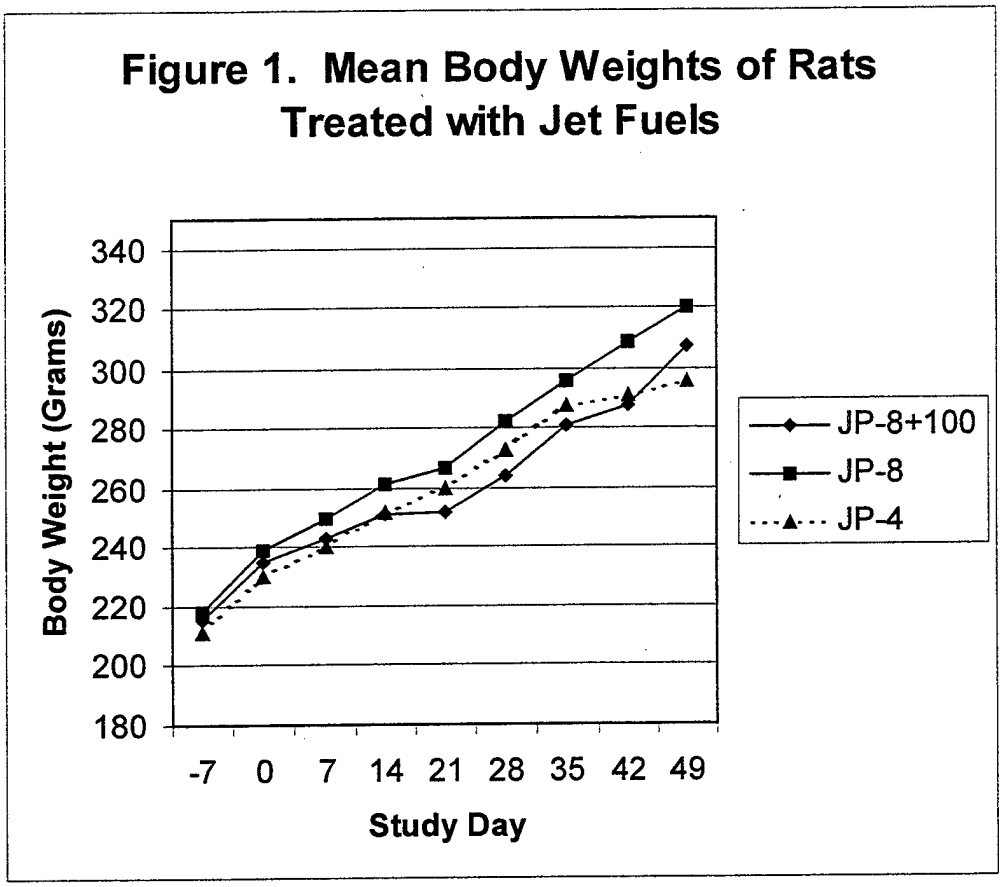
Clinical Observations (Non-Skin), Body Weights, and Organ Weights

There were no clinical signs of irritancy or toxicity unrelated to the skin. Body weight means were similar for all jet fuel groups throughout the study (Table 4 and Figure 1). Body weight gains appeared similar among groups and did not differ between the dosing period (Study Days 0 through 27) and the post-dosing (recovery) period (Study Days 28 through 49).

Table 4. Body Weights of Rats Treated with Jet Fuels

Study Day (Week)	Study Phase	Group Size (n)	JP-8+100	JP-4	JP-8
-7 (-1)	Pre-study	14	215 ± 8[a]	211 ± 7	218 ± 6
0 (0)	1st Day of Dosing	14	235 ± 9	230 ± 8	239 ± 6
7 (1)	8th Day of Dosing	14	243 ± 10	240 ± 10	250 ± 6
14 (2)	15th Day of Dosing	12	251 ± 11	252 ± 10	261 ± 6
21 (3)	22nd Day of Dosing	10	252 ± 13	260 ± 9	267 ± 5
28 (4)	1st Day of Recovery	8	264 ± 15	273 ± 10	282 ± 6
35 (5)	8th Day of Recovery	6	281 ± 18	288 ± 9	296 ± 7
42 (6)	15th Day of Recovery	4	288 ± 20	291 ± 5	309 ± 7
49 (7)	22nd Day of Recovery	2	307 ± 29	296 ± 4	320 ± 5

[a]Mean ± SD, grams



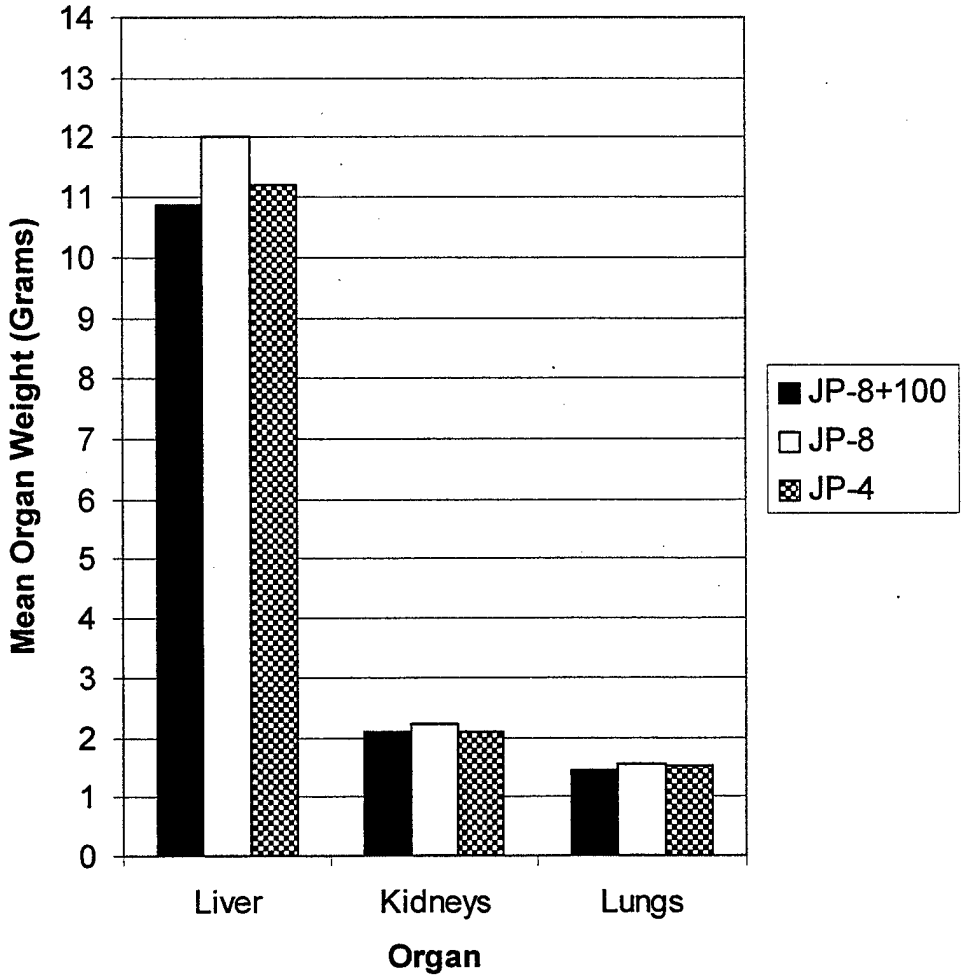
Organ weight means and ranges were similar for all jet fuel groups (Table 5 and Figure 2). Organ weights did not appear to differ between animal termination periods within a jet fuel group (data not shown). Thus, organ weight means and ranges were calculated on all animals within a treatment group (Table 5).

Table 5. Organ Weights of Rats Treated with Jet Fuels

Organ	JP-8+100	JP-4	JP-8
Liver	10.86 ± 1.02[a]	11.20 ± 1.49	12.00 ± 1.16
	(9.19 – 12.89)[b]	(8.69 – 14.07)	(10.04 – 13.78)
Kidneys	2.10 ± 0.14	2.08 ± 0.17	2.22 ± 0.13
	(1.92 – 2.27)	(1.76 – 2.36)	(2.06 – 2.46)
Lungs	1.44 ± 0.09[c]	1.53 ± 0.16[c]	1.55 ± 0.12
	(1.30 – 1.63)[c]	(1.32 – 1.78)[c]	(1.41 – 1.77)

[a]Mean ± SD, grams, n = 14
[b]Range of individual animal organ weights, grams, n = 14
[c]n = 13

Figure 2. Organ Weights of Rats Treated with Jet Fuels



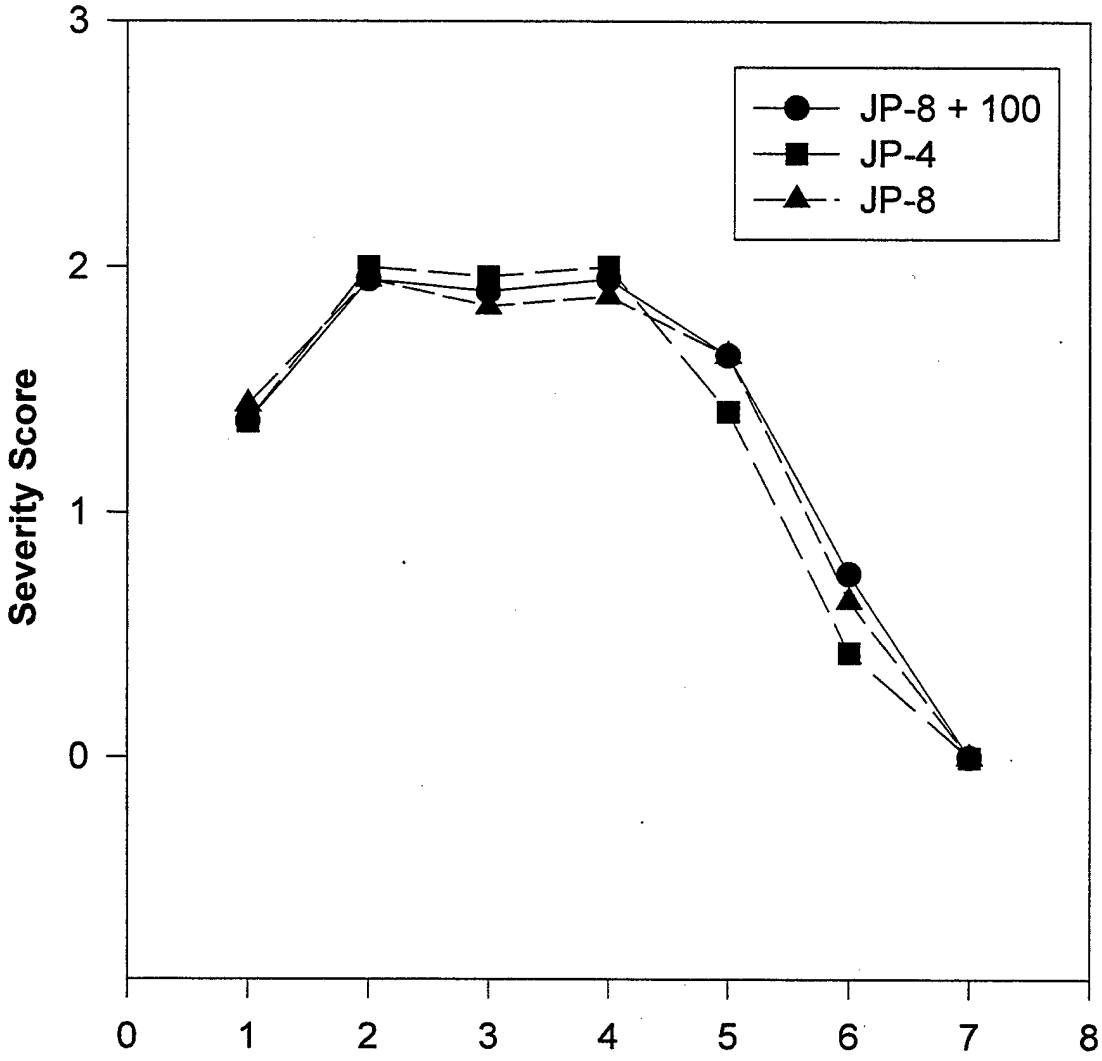
Clinical Observations of the Skin

Individual animal scores of gross lesions (erythema, edema, flaking, lichenification, and fissure formation) are presented in tabular form in Appendix A. Weekly average severity scores for erythema and edema are summarized graphically in Figures 3a and 3b, respectively. A narrative description of these changes follows.

Gross lesions (i.e., erythema, edema, and flaking/fissuring of skin) were noted daily during the 7-week study. All groups demonstrated similar responses with lesions progressing within the first week to levels sustained throughout the exposure phase. Erythema or redness of skin at the treatment site was only slight (pale pink) for the first 3 days of the first week. Toward the end of that week and for the remaining exposure time, these regions were a well-defined dark pink. Edematous changes followed a similar pattern with barely perceptible swelling noted for the first five days followed by slight edema with well defined, elevated edges noted throughout the exposure phase and for the first week of the recovery phase. Flaking of the skin at exposure sites was evident by the fourth day of Week 1 of the exposure phase. Flaking subsided or was masked by other changes by day 25 in all groups, but was once again noted in the JP – 4 group between days 15-32. By the end of the first week, the first signs of lichenification or roughened thickening of the skin were noted in all groups. This change persisted throughout the remainder of the exposure phase and was resolved in all groups during the second week of the recovery phase. Fissures of the epidermis are variably deep cracks in the skin which expose the subjacent dermis. This change promotes loss of serocellular fluid from these sites with the ultimate formation of a crust over the adjacent thickened skin. Fissuring was evident in all groups by day 7 of the first week of exposure. During the following 3 weeks fissuring with crust formation was a dominant change. Upon withdrawal of exposures, this condition began to resolve with complete resolution being attained in all groups between the third day of Week 1 and the second day of Week 2 of the recovery phase.

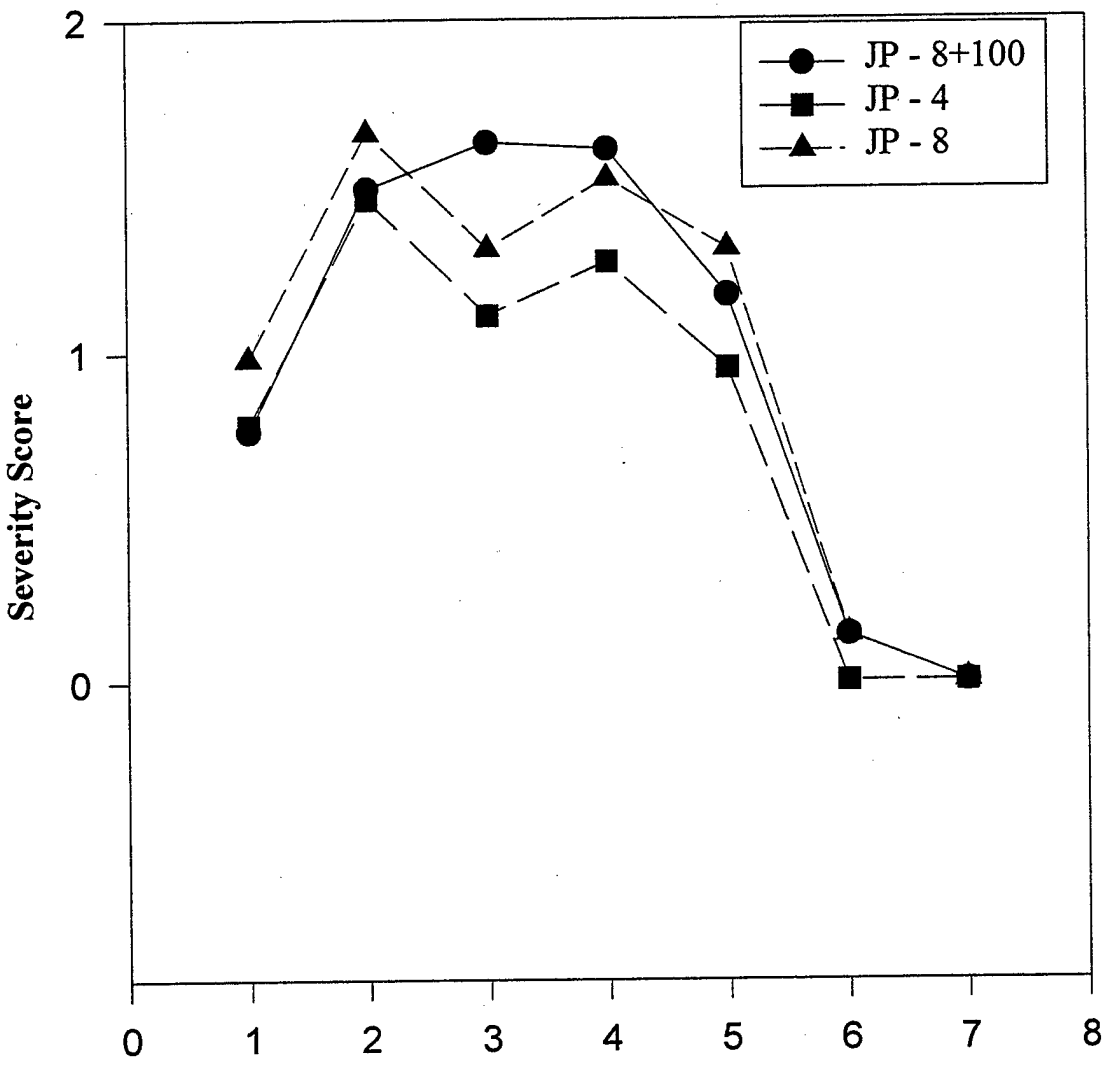
Figure 3a

Mean Weekly Severity Scores for Erythema (Gross Observations)



Exposure Phase-Weeks 1-4--/ /--Recovery Phase-Weeks 5-7
Observations per time point were 14, 12, 10, 8, 6, 4, & 2 for Weeks 1-7, respectively

Mean Weekly Severity Scores for Edema (Gross Observations)



Exposure Phase-Weeks 1-4--/ /--Recovery Phase-Weeks 5-7
Observations per time point were 14, 12, 10, 8, 6, 4, & 2 for Weeks 1-7, respectively

Figure 3b

Histopathology of the Skin

Scores of microscopic lesions were recorded on assessment sheets included in Appendix B. Mean histopathologic assessment scores are presented in Table 6 below, as well as graphically in Figure 4. Also included below are lesion incidence tables (Tables 7a and 7b). Tables 8a and 8b display mean severity scores for each lesion computed for each treatment group's exposure phase and recovery phase. Statistical comparisons of microscopic findings were made using Sigma Stat by Statistical Product and Service Solutions (SPSS), Chicago, IL.

Microscopic assessments represent the cumulative effects of agent exposures by weekly intervals. Increased histopathologic assessment scores for skin lesions were observed following the first week of exposure and continued throughout the 4-week exposure phase. Termination of exposures was associated with a rapid return of epidermal and dermal elements to normal gross and microscopic appearances. Photomicrographs of histologic sections of treated, untreated, and recovered skin samples are provided in Figures 5a, b, and c. As in the pilot study (Baker *et al.*, 1999), all microscopic characteristics that assessed epidermal thickness, whether by direct measurement or counts of layers of epidermal keratinocytes and basal cells, accounted for the greatest proportion of the dermal response score. The average thickness of the epidermis in control samples was 13.01 *u*m (n=42), whereas, in the JP-8 + 100, JP-4, and JP-8 groups during the exposure phase, thicknesses were 48.78$\pm$16.27 (n=8), 40.23$\pm$6.21 (n=8), and 49.05$\pm$12.48 (n=8) *u*m, respectively. Additionally, many other changes contributed in a minor way to overall score, and are directly related to epidermal thickness; for example, scores associated with proliferative changes (hyperkeratosis, hyperplasia, and mitoses/4mm). Scores reflecting inflammatory change (i.e., inflammatory infiltrates, edema, and vasodilatation), contributed to the cumulative score, as did degenerative changes (erosion, ulceration, and acantholysis). Crust formation is a product of encrusted serum mixed with cellular debris, bacteria, and keratin and was limited to lesions in which the subcuticular region had been exposed by breaks in the overlying epithelium. During the exposure phase, microscopic observation of ulceration (or break in the epidermal basement membrane) occurred in a limited number of animals (4/24, 16.7%), whereas crust formation was more prevalent (10/24, 41.7%), suggesting that ulceration was underdiagnosed in the histopathologic assessment. This is not surprising in that the histosectioning process is more apt to include the wide spread encrustation than the focal rent in the epithelium. Changes indicative of increased epidermal cell proliferation, hyperkeratosis (ortho- and parakeratosis), hyperplasia, and hypergranulosis, were observed with greater frequency during the exposure phase. Epidermal hyperplasia, noted in all exposure phase samples, is a nondiagnostic feature of virtually any chronic inflammatory process. Spongiosis, intercellular edema of the epidermis, noted in 50% of the samples is, likewise, a nonspecific, inflammatory change. Although the term inflammatory process evokes an image of cellular infiltrates, inflammatory infiltrates were uncommonly noted in these samples. Their presence was most conspicuous in areas adjacent to ulceration. Likewise, vasodilation and edema within the dermis were not limited to regions of cellular infiltrates. These observations suggest that proliferative and vascular changes may have been, in part, due to direct or indirect stimulation of epidermal and dermal elements by the test agents and not only secondary to inflammation induced by these agents. On the basis of lesion frequency, hydropic degeneration was noted in 73.8% of control samples and therefore did not serve as discriminating characteristics in this study.

For statistical comparisons, all 42 control observations were combined for increased statistical power. The combined mean control score was 16.8± 3.6. In that there were only 2 observations per treatment week per exposure, the 8 observations per exposure group were combined for statistical comparisons. The mean scores for the 3 treatment groups during the four-week exposure phase [69.3±24.4 (n=8), 58.9±7.8 (n=8), and 69.5±20.0 (n=8) for JP-8+100, JP-4, and JP-8, respectively] were significantly higher than control scores, with P values less than 0.0001 using a t-test or the Mann-Whitney Rank Sum Test. Treated in a similar fashion, mean scores for the 3 groups during the three-week recovery phase [20.1± 4.2 (n=6), 19.7±2.7 (n=6), and 21.6±7.0 (n=6) for JP-8+100, JP-4, and JP-8, respectively] did not differ from control scores. Mean exposure phase scores, presented above, demonstrated no differences when compared with one another. Likewise, no differences were noted between the mean recovery phase scores. Although the mean exposure phase scores were not significantly different from one another, the mean scores for JP-8+100 and JP-8 were greater than the score for JP-4. This relationship is consistent with scores observed in the pilot study in which JP-4 severity scores were less than those of JP-8 (Baker *et al.*, 1999). This observation is also consistent with anecdotal accounts of human exposures in which JP-8 has been reported as a greater irritant than JP-4.

Table 6. Mean Histopathologic Assessment Scores

Treatment Week	JP-8+100		JP-4		JP-8		Mean Control Score By Exposure Week
	Treated Score	Mean Score	Treated Score	Mean Score	Treated Score	Mean Score	
	64.2		46.8		105.8		
1	83.4	**73.8**	72.2	**59.5**	82.2	**94.0**	**17.2 n=6**
	84.4		59.6		60.0		
2	54.8	**69.6**	57.0	**58.3**	58.2	**59.1**	**13.8 n=6**
	97.6		58.8		42.4		
3	87.0	**92.3**	56.4	**57.6**	59.8	**51.1**	**20.3 n=6**
	21.0		53.2		62.8		
4	61.8	**41.4**	66.8	**60.0**	84.8	**73.8**	**20.1 n=6**
Exposure Phase							
Means		**69.3** **n=8**		**58.9** **n=8**		**69.5** **n=8**	**16.2 n=24**
St Dev		**24.4**		**7.8**		**20.0**	**3.4**
	23.0		20.6		18.8		
5	26.0	**24.5**	19.2	**19.9**	32.4	**25.6**	**18.9 n=6**
	17.8		16.8		18.2		
6	14.4	**16.1**	16.6	**16.7**	26.2	**22.2**	**16.7 n=6**
	21.2		23.6		21.8		
7	18.0	**19.6**	21.2	**22.4**	12.4	**17.1**	**17.5 n=6**
Recovery Phase							
Means		**20.1** **n=6**		**19.7** **n=6**		**21.6** **n=6**	**17.7 n=18**
St Dev		**4.2**		**2.7**		**7.0**	**3.8**

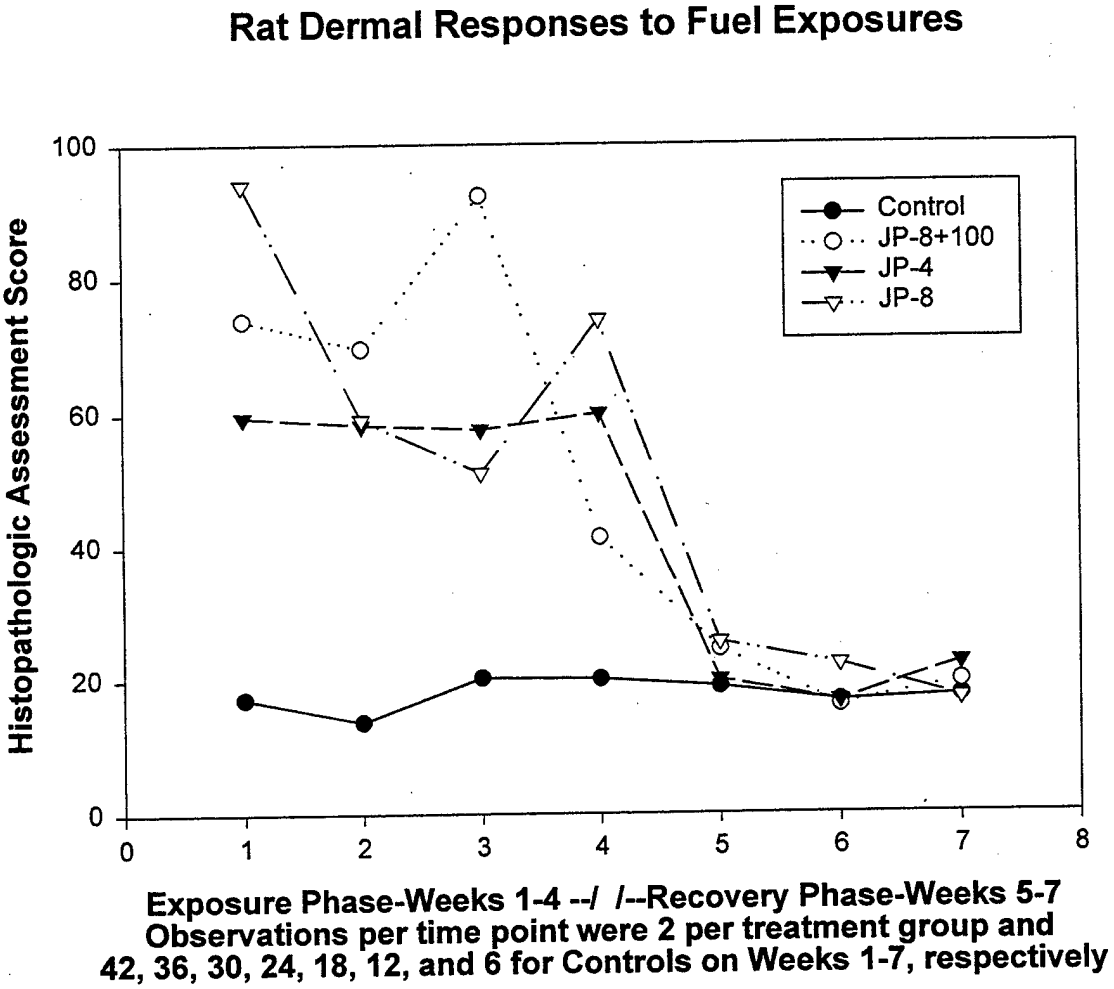

Rat Dermal Responses to Fuel Exposures
Histopathologic Assessment Score
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Control
JP-8+100
JP-4
JP-8
Exposure Phase-Weeks 1-4 --/ /--Recovery Phase-Weeks 5-7
Observations per time point were 2 per treatment group and
42, 36, 30, 24, 18, 12, and 6 for Controls on Weeks 1-7, respectively

Figure 4

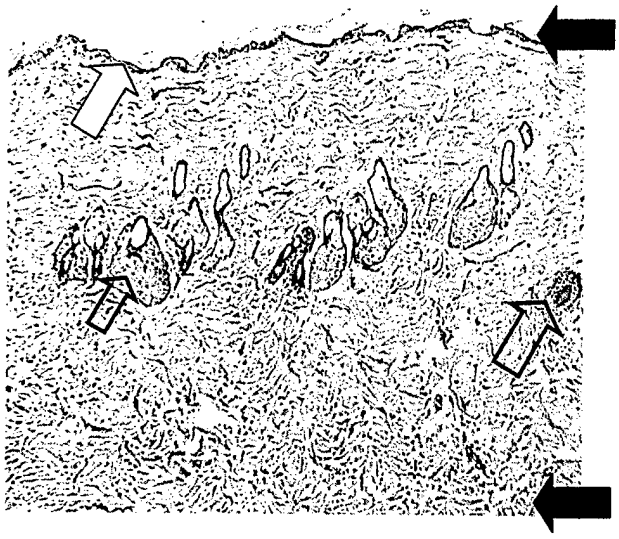


Figure 5a. Control (Animal # 424-98B)

A section of rat skin at 10X magnification demonstrating the normal characteristics and spatial relationships of epidermis (white arrow), dermis (between black arrows), and dermal adnexa [hair follicles (open large arrows) and sebaceous gland (open small arrow)]. Treated skin from the same animal is pictured below.

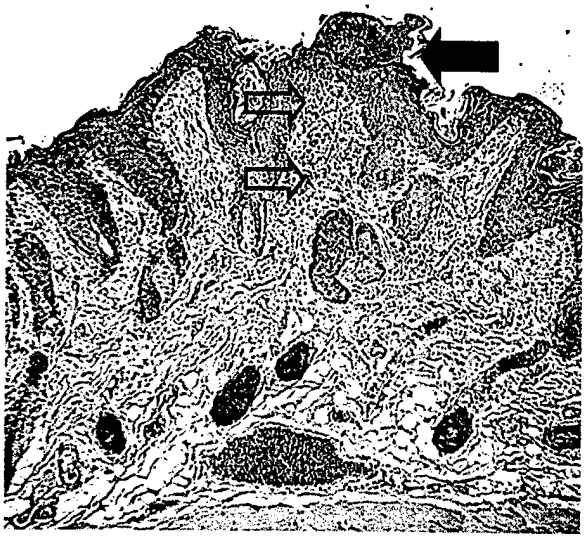


Figure 5b. Treated, Wk 3 (Animal # 424-98A)

10X Magnification. This is a sample of skin topically treated 1x/day for 3 weeks with neat JP-8. Interfollicular and follicular epidermis is dramatically thickened. There is a prominent focus of serocellular crust (closed arrow), below which the epidermis is slightly eroded. The subjacent dermis displays an inflammatory focus with a minimal accumulation of lymphocytes admixed with occasional neutrophils (between open arrows). The deep dermal vessel at bottom center is markedly dilated.

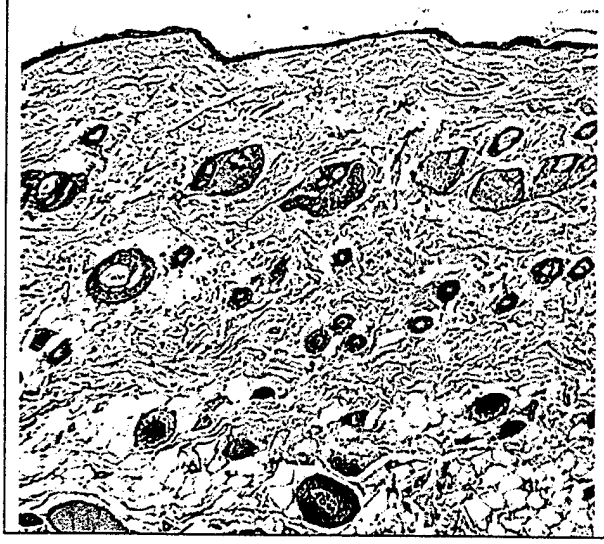


Figure 5c. Treated, Wk 7 (Animal # 448-98A)

10X Magnification. This sample, treated 1x/day for 4 weeks with neat JP-8 and allowed to recover for 3 weeks, appears to be essentially normal tissue.

Table 7a. Frequencies of Lesions-Exposure Phase

	Controls	JP-8+100	JP-4	JP-8
Erosion	0/42	1/8	0/8	1/8
Ulceration	0/42	1/8	1/8	2/8
Crust Formation	0/42	5/8	3/8	2/8
Epidermitis	0/42	2/8	0/8	1/8
Necrosis	0/42	1/8	1/8	1/8
Acantholysis	0/42	2/8	0/8	1/8
Spongiosis	0/42	4/8	4/8	5/8
Hydropic Degeneration	31/42	7/8	7/8	8/8
Orthokeratosis	0/42	7/8	8/8	7/8
Parakeratosis	0/42	5/8	5/8	6/8
Hyperplasia	1/42	8/8	8/8	8/8
Hypergranulosis	1/42	7/8	8/8	8/8
Dyskeratosis	0/42	3/8	4/8	2/8
Inflammatory Infiltrates	0/42	6/8	3/8	3/8
Edema	0/42	4/8	1/8	3/8
Vasodilation	0/42	7/8	5/8	7/8

Table 7b. Frequencies of Lesions-Recovery Phase

	Controls	JP-8+100	JP-4	JP-8
Erosion	0/42	0/6	0/6	0/6
Ulceration	0/42	0/6	0/6	0/6
Crust Formation	0/42	0/6	0/6	0/6
Epidermitis	0/42	0/6	0/6	0/6
Necrosis	0/42	0/6	0/6	0/6
Acantholysis	0/42	0/6	0/6	0/6
Spongiosis	0/42	0/6	0/6	0/6
Hydropic Degeneration	31/42	4/6	5/6	4/6
Orthokeratosis	0/42	0/6	0/6	1/6
Parakeratosis	0/42	0/6	0/6	0/6
Hyperplasia	1/42	0/6	1/6	1/6
Hypergranulosis	1/42	4/6	2/6	3/6
Dyskeratosis	0/42	0/6	2/6	0/6
Inflammatory Infiltrates	0/42	0/6	1/6	1/6
Edema	0/42	0/6	0/6	0/6
Vasodilation	0/42	0/6	0/6	0/6

Table 8a. Mean Lesion Severity Score-Exposure Phase

	Controls	JP – 8 + 100	JP – 4	JP - 8
Erosion	0	1	0	1
Ulceration	0	1	1	0.38
Crust Formation	0	0.75	0.38	0.38
Epidermitis	0	0.25	0	0.13
Necrosis	0	0.13	0.13	0.13
Acantholysis	0	0.38	0	0.25
Spongiosis	0	0.63	0.63	0.88
Hydropic Degeneration	0.74	1.00	0.88	1.00
Orthokeratosis	0	1.50	1.25	1.63
Parakeratosis	0	1.25	0.75	1.00
Hyperplasia	0.02	1.25	1.00	1.25
Hypergranulosis	0.02	0.88	1.00	1.00
Dyskeratosis	0	0.38	0.50	0.25
Epidermal Thickness (*um*)	13.01	48.78	40.23	49.05
Average Cells/100 *um*	2.40	6.13	6.25	7.08
Mitoses per 4 mm	0.64	1.38	4.00	2.25
Inflammatory Infiltrates	0	0.88	0.75	0.50
Edema	0	1.13	0.13	0.75
Vasodilation	0	2.38	0.88	1.50

Table 8b. Mean Lesion Severity Score-Recovery Phase

	Controls	JP – 8 + 100	JP – 4	JP - 8
Erosion	0	0	0	0
Ulceration	0	0	0	0
Crust Formation	0	0	0	0
Epidermitis	0	0	0	0
Necrosis	0	0	0	0
Acantholysis	0	0	0	0
Spongiosis	0	0	0	0
Hydropic Degeneration	0.74	0.75	0.83	0.66
Orthokeratosis	0	0.00	0.00	0.17
Parakeratosis	0	0.00	0.00	0.00
Hyperplasia	0.02	0.00	0.17	0.17
Hypergranulosis	0.02	0.66	0.33	0.50
Dyskeratosis	0	0.00	0.33	0.00
Epidermal Thickness (*um*)	13.01	15.17	13.97	15.10
Average Cells/100 *um*	2.42	2.70	3.20	2.50
Mitoses per 4 mm	0.64	0.50	1.17	1.67
Inflammatory Infiltrates	0	0	0.17	0.17
Edema	0	0	0	0
Vasodilation	0	0	0	0

REFERENCES

Baker, W., English, J., Dodd, D., McDougal, J., and Miller, T. (1999). Repeated dose skin irritation study on jet fuels – preliminary dose range finding study. AFRL-HE-WP-TR-1999-0008.

Bruner, RH. Pathological Processes of Skin Damage Related to Toxicant Exposure, pp 73-109, In: *Dermal and ocular toxicology: fundamentals and methods*. Ed. David W. Hobson, CRC Press, Inc., Boca Raton, FL, 1991.

Draize, J.H., Woodard, G., and Calvery, H.O., (1944). Method for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. 82:377-390.

EPA Health Effects Testing Guidelines, section 798.4470, Primary dermal irritation. 40 CFR (7-1-90 edition)

Eurell, T.E. (1994). Effects of three hydrocarbons on the histologic structure of male rat kidneys. AFOSR-TR-0616

Harris, D.T. (1995). Immunotoxicity of jet fuel. AFOSR-TR-96-0156

Modiano, S.H., McNesby, K.L., Marsh, P.E., Bolt, W., and Herud, C. (1996). Quantitative measurement by Fourier Transform Infrared (FT-IR) spectroscopy of toxic gas production during inhibition of JP-8 fires by CF3Br and C3F7H. ARL-TR-990

U.S. Air Force. (1992). MILITARY SPECIFICATION TURBINE FUEL, AVIATION, KEROSENE TYPES, NATO F-34 (JP-8) AND NATO F-35. MIL-T-83133D. Wright-Patterson AFB, OH 45433.

Witten, M.L. (1994). Chronic effects on JP-8 jet fuel exposure on the lungs. AFOSR-91-0199

Wolfe, R.E., Kinkead, E.R., Feldmann, M.L., Leahy, H.F., Jederberg, W.W., Mattie, D.R., and Still, K.R. (1996). Acute toxicity evaluation of JP-8 jet fuel and JP-8 jet fuel containing additives. AL/OE-TR-1996-0136